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# Endophytic Bacillus: the Potentiality of Antagonism to Wilt Pathogen and Promoting Growth to Micro-Plantlet of Banana in Vitro

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**ABSTRACT**

Using healthy seedling produced by tissue culture is one of the recommended control strategies of banana wilt diseases caused by blood disease bacterium (BDB) and *Fusarium oxysporum* f.sp. *cubense* (FOC) in Indonesia. The tissue culture technique however may cause the seedling to be susceptible to the diseases due to the aseptic work throughout producing, being able to eliminate the beneficial endophytic bacteria. Therefore it is hopeful that revitalizing the beneficial bacteria on the seedling will be able to recover the resistance of banana. Herewith, an amount of 10 isolates of endophytic Bacillus had been studied on the antagonism to the wilt pathogens and the ability to promote growth of banana micro-plantlet in vitro. The present study showed that (1) Bacillus isolate B04, B05, and B10 were antagonistic to BDB and FOC, (2) Bacillus isolate B04, B05, and B10 could release extracellular compound being able to promote the growth of banana micro-plantlets, and (3) Bacillus filtrate treatment of banana micro-plantlet was better than those infestation treatment into culture medium in promoting the growth of the micro-plantlet.

## Introduction:

Banana Wilt disease is an important constraint in cultivation of banana in Indonesia. Banana wilt disease is caused by blood disease bacterium (BDB) and or *Fusarium oxysporum* f.sp. *cubense* (FOC). In the fields, the disease incidence could reach over 90% [9]. Co-infection of the couple pathogen on individual banana were most frequent in the fields so the effect becomes more severe [7]. So far, the disease is still difficult to control. The use of healthy seedling is an important component in integrated management of the banana wilt disease. In the effort to provide pathogen free seedlings had been developed through producing with tissue culture technology [11, 14].

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Smith et al.[12] reported that banana seedling produced by tissue culture was susceptible to wilt pathogen caused by the aseptic conditioning work throughout culture process in vitro. Presumably, some beneficial endophytic associated the resistance of banana were eliminated.

Bacillus is one the genus of endophytic bacteria being effective as biological control agents A part of the genus lives in most plants that is associated with promoting plant growth, inducing plant resistance, because the bacterium produces indole acetic acid like substances (IAAS), dissolving phosphate, producing siderophore, and antibiotic [5, 15]. Therefore, the exploration, and potentiating endophytic *Bacillus* spp. of banana as the agents of promoting and inducing resistance of banana to the wilt diseases is an interesting study. This article reports the results of the test of antagonism and promoting growth of Bacillus to banana micro-plantlets.

## Material and Methods:

### Isolates of Bacillus:

Bacillus isolates were isolated from 3 peaces ±(0,2 x 0,5 x 10 mm) the bit of internal tissues of peduncle, pseudostem, and rhizome dipped in 5 ml sterile water. The samples were incubated for 12 hours and shaken with Vortex Shaker. A volume of 100 µl suspension was plated on Nutrient Agar (NA) with spread technique on medium in the

dish with composition 8 g Nnutrient Broth and 15 g agar Bacto in 1 L waters. The dishes were incubated for 24 hours. Single colony was sub-cultured using the same medium. The pure isolates were streaked on aslant NA medium for stock.

**Antagonistic test of Bacillus to BDB in vitro:**

Pathogen suspension with density $10^7$-$10^9$ cfu/ml mixed and homogenized in NA medium near before being solid in Petri dish Ø9 cm. The solid medium was placed 4 pieces of filter paper Ø0.5 cm having been dipped with bacterial suspension the antagonist candidate with density 108 cfu/ml. The filter papers were positioned in proportionally at the same away from each other, then in the center of medium was placed a piece of filter paper dipped in sterile water as a control. After incubating for 48-72 hours, the zone of inhibition caused by the antagonist candidate was observed. The test was arranged using completely randomized design with 3 replications. Obtained data were analyzed with Duncan's Multiple Rang Test.

**Antagonistic test of Bacillus to FOC in vitro:**

Antagonistic test of Bacillus to FOC was done with dual culture technique. Bacillus isolates were streaked on Potato Dextrose Agar (PDA) in Petri Dish Ø 9 cm, with 3 cm away from the point where FOC inoculants was placed. The cultures were incubated for 5 days. Antagonistic interaction was evaluated with accessing diameter of the inhibition zone. The percentage of inhibition zone was accessed using formula of 1-(a/b) x 100%, where a: the distant between center colony of FOC directing to Bacillus, and b the distant between center colony of FOC directing to empty area with no Bacillus. The tests were arranged with completely randomized design with 3 replication. Obtained data were analyzed with Duncan's Multiple Range Test.

**Test of growth promoting activity of Bacillus to micro-plantlet:**

Microplantlet at 6 months of age, was planted in culture bottle containing 30 ml modified Murashige and Skoog medium[11] added charcoal powder 0,5 g/L and 30 g dextrose [14]. A piece of shoot of micro-plantlet was ± 3 cm in the length. Before panting, the micro-plantlet was inoculated by dipping with $10^8$ cfu/ml or 5% filtrate of Bacillus culture in the medium. The cultures were incubated at 23-28$^o$C and the micro-plantlet was incubated for 3 weeks. Observation was done to access the sum of shoot, high of shoot, and sum of leaf.

## Result and Discussion:

Ten isolates of endophytic bacterium from healthy banana in endemic area of BDB and FOC had been selected. The results of antagonism test showed that all of the isolates were antagonistic to the couple pathogen (Table 1). Three isolates of Bacillus (B04, B05, and B10) are the most effective in inhibiting the growth of pathogen.

The present results are in accordance with previous evident that an apart from the genus of *Bacillus* spp. is a potential biocontrol agent of plant diseases caused by bacteria and fungi [1, 2, 3, 4, 5, 6, 8, 10, 15]. Brooks et al [4] reported that 183 isolates of 889 endophytic bacteria isolates from Oak were antagonistic to wilt pathogen, *Ceratocystis fagacearum* in vitro. Chen et al [6] also reported that 10 isolates of entophytic bacterium could suppressed disease severity of cotton wilt caused by *Fusarium oxysporum* f.sp. *vasinfectum* through inoculating the seedling. Benhamou et al.(2) added that *B. pumilus* race SE 34 could induce plant resistant of potato to wilt disease caused by *Fusarium oxysporum* f.sp. *radicis-lycopersici.*

**Table 1: Effect culture filtrates of Bacillus to the growth of micro-plantlet on MS medium**

| Isolate of Bacillus | Zone of inhibition (mm) | |
|---|---|---|
| | BDB* | FOC* |
| 1. **B01** | 6.2b | 1.3bc |
| 2. **B03** | 5.1bc | 1.4bc |
| 3. **B04** | **14.1a** | **3.6a** |
| 4. **B05** | **12.3a** | **3.3a** |
| 5. **B06** | 7.3b | 2.7b |
| 6. **B07** | 6.5b | 1.3bc |
| 7. **B10** | **13.4a** | **3.1a** |
| 8. **B15** | 5.3bc | 1.5bc |
| 9. **B16** | 3.2c | 0.4c |
| 10. **B17** | 4.4c | 0.7c |
| 11. **Control** | 0.0d | 0.0d |

*the average marked by the same letter is not significantly different based on Duncan's Test (P≤0.05)

### Effect of filtrates and infested medium by Bacillus to the growth of micro-plantlet of banana:

The results showed that the filtrate of 3 isolates of Bacillus could promote the growth of banana micro-plantlets (Table 2). The isolates are B04, B05, and B10. The same trend was performed by the results tests of Bacillus infestation into culture medium that the 3 isolates were could promote the growth of banana micro-plantlets. It supports the previous researches explaining that *Bacillus* spp. could be plant growth promoter due to by the capability of the microbe to produce IAA, release siderophore, and take a role as biological control agents through inducing systemic resistance of plant and release antimicrobial[2, 4, 5, 15].

**Table 2: Effect of culture filtrate of Bacillus to the growth of micro-plantlet on MS Medium**

| Isolate of Bacillus | Micro-plantlet life (%) | Number of Shoot | High of Shoot (cm)* | Number of Leaf* |
|---|---|---|---|---|
| 1. B01 | 100 | 2.00 | 9.50ab | 2.31c |
| 2. B03 | 100 | 2.66 | 8.28bc | 2.81c |
| 3. B04 | **100** | **3.66** | **11.61a** | **7.80a** |
| 4. B05 | **100** | **4.33** | **11.32a** | **6.03ab** |
| 5. B06 | 100 | 3.33 | 9.06ab | 3.06c |
| 6. B07 | 100 | 3.66 | 9.61ab | 3.45c |
| 7. B10 | **100** | **5.00** | **11.60a** | **5.21abc** |
| 8. B15 | 100 | 4.66 | 10.96abc | 3.10c |
| 9. B16 | 100 | 2.33 | 9.40ab | 4.22bc |
| 10. B17 | 91.67 | 2.66 | 8.56bc | 2.54c |
| 11. Control | 100 | 2.33 | 9.36ab | 3.40c |
| **Average** | **99.243** | **3.296±1,07$^{ns}$** | **9.933±1.22** | **3.971±1.79** |

*the averages marked by the same letter are not significantly different based on Duncan's Test (P≤0.05)ns all of the averages are not significantly different based on the variance analysis (P≤0.05).

In comparing between Table 2 and Table 3 show that the growth of banana micro-plant cultured on the medium infested by Bacillus were lower than those on medium with the filtrate of Bacillus. Possibly, it was caused by nutrition competition between micro-plantlet and Bacillus. Suggesting the results and discussion that Bacillus isolate B04, B05 and B10 should be studied further for biological control agents of BDB and FOC in addition as plant growth promoter bacteria on the seedling of Banana producing with tissue culture technology.

**Table 3: Effect of Bacillus infestation into culture medium to the growth of micro-plantlets on MS Medium**

| Isolate of Bacillus | Micro-plantlet Life (%) | Number of Shoot | High of Shoot (cm)* | Number of Leaf* |
|---|---|---|---|---|
| 1. B01 | 100 | 2.66 | 8.35c | 2.02c |
| 2. B03 | 100 | 2.33 | 8.01c | 2.98bc |
| 3. B04 | **100** | **3.33** | **9.41ab** | **4.70ab** |
| 4. B05 | **100** | **4.00** | **11.42a** | **5.30a** |
| 5. B06 | 100 | 3.00 | 8.05c | 3.05ab |
| 6. B07 | 100 | 3.33 | 8.18c | 3.85ab |
| 7. B10 | **100** | **4.33** | **10.44ba** | **4.31ab** |
| 8. B15 | 100 | 3.33 | 9.72ab | 3.10abc |
| 9. B16 | 100 | 3.00 | 8.93bc | 3.44abc |
| 10. B17 | 100 | 3.66 | 8.63bc | 3.22abc |
| 11. Control | 100 | 2.66 | 9.46ab | 2.38bc |
| **Average** | **100** | **3.323±0.58$^{ns}$** | **8.963±0.79** | **3.486±0.98** |

*the averages marked by the same letter are not significantly different based on Duncan's Test (P≤0.05) ns all of the averages are not significantly different based on the variance analysis (P≤0.05)

**Conclusion:**

The present study showed that: (1) Bacillus isolates E B04, B05, and B10 were antagonistic to BDB and FOC, (2) Bacillus isolate B04, B05, and B10 could release extracellular compound being able to promote the growth of micro-plantlets, and (3) Filtrate of Bacillus treatment of micro-plantlet was better than those infestation treatment into culture medium in promoting of the micro-plantlet.

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